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A randomized clinical trial on the anti-tumoral effects of low molecular weight heparin in the treatment of esophageal cancer

Running title: antineoplastic effects of LMWH

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Abstract

Current treatment approaches for esophageal cancer are associated with a poor survival, and there are ongoing efforts to find new and more effective therapeutic strategies. There are several reports on the anti-tumoral effects of low molecular weight heparin (LMWH). We have assessed the possible survival benefit of LMWHs in esophageal malignancies. This was a randomized, single blind, multi-center, phase II clinical trial on non-metastatic esophageal cancer candidate for neoadjuvant chemoradiotherapy. Patients were randomly assigned to the chemoradiotherapy-only arm or chemoradiotherapy plus enoxaparin arm using 1:1 allocation. Radiotherapy was delivered in 1.8-Gy daily fractions to a dose of 50.4 Gy in both groups. Paclitaxel 50 mg/m2 and carboplatin (AUC 2) were administered weekly concurrent with radiotherapy. In the intervention group, patients received enoxaparin (40 mg) daily as well as chemoradiation. Four to six weeks after treatment, all patients underwent esophagectomy. After a median follow up of 7 months, estimated one year disease free survival (1y DFS) in the intervention group was 78.9% and in the control groups was 70% (p=0.5). Toxicity from the experimental treatment was minimal and there were no treatment-related deaths. A Pathologically complete response in intervention and control group was 64.8% and 62.5%, respectively (p=0.9). There was a non-significant trend toward improved survival by the addition of enoxaparin to the concurrent chemoradiotherapy regimen. However, 1y DFS of both groups were high as expected. A longer follow-up and larger sample size is required.

1. Background

Currently, a multimodal approach (neoadjuvant chemotherapy and/ or radiation therapy, followed by esophagectomy) is considered to be the standard of care for the treatment of thoracic esophageal cancers; however, 5-year survival rates remain poor at between 12–20% (De Angelis et al., 2014; Siegel et al., 2016). Neoadjuvant treatment leads to an increased likelihood of complete surgical resection (R0 resection), improved local control and modestly ameliorates disease-specific and overall survival; it also allows an evaluation of the effectiveness of the treatment regimen for each clinical scenario (Posner et al., 2015). The most contemporary published randomized controlled trials (RCT) that inform current clinical practice are the: ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS) (Shapiro et al., 2015), Medical Research Council (MRC) Adjuvant Gastric Infusional Chemotherapy (MAGIC) (Cunningham et al., 2006), the Fédérale Nationale des Centres de Lutte Contre Le Cancer (FNLCC) ACCORD (Ychou et al., 2011), and MRC OEO2 (Allum et al., 2009; Medical Research Council Oesophageal Cancer Working Group, 2002) studies. Currently, phase II and III trials focus on determination of the optimal components of the neoadjuvant therapies that lead to introduction of targeted therapies and immune based approaches in the treatment of esophageal cancer (Anvari et al., 2017; Bang et al., 2010). One agent that has attracted recent attentions, is low molecular weight heparins (LMWHs) that has been historically used for treatment of cancer induced deep venous thrombosis (DVT) (National Comprehensive Cancer Network, 2011). There are several reports on the anti-tumoral effects of LMWH. We have assessed the possible survival benefit of LMWHs in esophageal malignancies.

2. Methods

2.1. Trial Design

This was a multicenter, stratified (with balanced randomisation [1:1]), parallel-group phase II clinical trial conducted at three sites in northeastern Iran: The Emam Reza Hospital, Omid Hospital, and Reza Radiotherapy and Oncology Center in Mashhad from July 2016 to February 2018. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (reference number: MUMS.fm.REC.1395.53). The ethical rules of research according to Helsinki Declaration were observed and all patients signed a written consent form. The protocol of study was registered in the Iranian Registry of Clinical Trials (IRCT2016070628814N1) and ClinicalTrials.gov (NCT03254511).

2.2. Participants

Eligible participants were adults aged ≥18 years with squamous cell carcinoma (SCC) of esophagus who met the eligibility criteria for neoadjuvant chemoradiation and definitive esophagectomy (non-metastatic SCC of thoracic esophagus). Patients also had to have adequate hematologic, renal, hepatic, and pulmonary function. Primary exclusion criteria were pregnancy and lactation, previous history of chemotherapy or chest-wall irradiation, history of major comorbidities (including liver or renal failure), adenocarcinoma or small cell carcinoma of esophagus, or the presence of synchronous cancer. Enoxaparin is contraindicated if the patient has a condition with a high risk of haemorrhage. Secondary exclusion criteria were patient's refusal to agree to an esophagectomy and Eastern Cooperative Oncology Group (ECOG) performance status score ≥3 during neoadjuvant treatment.

2.3. Staging

Before treatment, all patients underwent diagnostic and staging work-up. This included taking a history; physical examination; routine hematologic and biochemical tests; upper gastrointestinal endoscopy with histologic biopsy; and computed tomography of the neck, chest, and upper abdomen. In case of presence of suspected cervical lymph nodes, ultrasonography of the neck, with fine-needle aspiration was performed. Patients did not undergo esophageal endoscopic ultrasound with fine-needle aspiration because of the prohibitive costs of a US-guided endoscopic approach which limits their widespread availability in Iran.

2.4. Interventions

Patients were randomly assigned to receive neoadjuvant chemoradiation alone or neoadjuvant chemoradiation plus enoxaparin. Radiotherapy was prescribed with a total dose of 50.4 Gy that was given in 28 fractions of 1.8 Gy each, with 5 fractions administered per week. Chemotherapy was administered weekly concurrent with radiotherapy that was consisted of carboplatin targeted at an area under the curve of 2 mg per milliliter per minute and paclitaxel at a dose of 50 mg per square meter of body-surface area intravenously. Before injection of paclitaxel, all patients were premedicated with intravenous dexamethasone, chlorpheniramine, and ranitidine as well as standard antiemetic agents. In the chemoradiation plus enoxaparin group, patients received enoxaparin sodium 40 mg/ 0.2 ml subcutaneous injection once daily during chemoradiation. This dose was chosen based on a review of the literature (Javadinia et al., 2018). Patients were monitored closely for possible side effects of chemotherapy or enoxaparin. Within 4 to 6 weeks after completion of neoadjuvant treatment, patients underwent esophagectomy. During the waiting period for esophagectomy, patients underwent upper gastrointestinal endoscopy for the assessment of the clinical response of the tumor to the neoadjuvant treatment.

2.5. Outcomes

The primary endpoints were clinical response, pathological response and R staging. Clinical response was defined as complete clinical response (no tumoral lesion), significant clinical response (a substantial reduction in the size of tumor, more than 50%), and poor clinical response (persistent lesion or reduction size of lesion less than 50%), based on preoperative upper gastrointestinal endoscopy. Pathologic response was defined as pathologic complete response (no evidence of vital residual tumor cells) and persistent disease (presence of any degree of vital residual tumor cells) based on pathologic evaluation of esophagectomy specimen. Residual staging (R staging) was classified according to the report of surgery and pathology. Any gross residual disease was considered to be R2 resection while microscopically positive margins were considered to be R1 resection. If there was neither a gross residual disease nor a microscopically positive margin, the surgery was considered to be R0 resection. The secondary endpoint was disease free survival which was considered over the length of time after esophagectomy, that the patient survived without any signs or symptoms of esophageal cancer (local or distal recurrence). Common Terminology Criteria for Adverse Events (CTCAE) v5.0 was used for reporting the adverse events.

2.6. Sample size and randomisation

Sample size was determined as at least 40 patients in each group based on confidence interval (CI): 95%, power: 80%, and effect size 45%. For allocation of the participants, a computer-generated list of random numbers was used with blocked randomisation [1:1].

2.7. Statistical methods

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 20.0 by the Mann–Whitney U, the Chi-Square, and Fisher's exacts. The Kaplan–Meier method was used to estimate survival, with the log-rank test to determine significance. P value≤0.05 was considered significant.

3. Results

3.1. Characteristics of patient

Between June July 2016 to February 2018, 69 patients from three participating centers (two academic centers and one large non-academic teaching hospital) were randomly assigned to neoadjuvant chemoradiation plus enoxaparin arm (n=37) or chemoradiation alone arm (n=32). The Consolidated Standards of Reporting Trials (CONSORT) diagram summarizes patient status (Figure 1). Both groups were similar in term of age, gender, ECOG performance score, tumor grade, and location tumor. Table 1 summarizes the data on demographic and baseline disease characteristics.

3.2. Outcomes and estimation

For the assessment of clinical response, preoperative endoscopy was requested. One patient in each group refused to consent. Complete clinical response was reported in 14 out of 36 patients of the chemoradiation plus enoxaparin arm and 11 out of 31 patients of chemoradiation alone arm (p=0.5). All patients in both arms had an R0 resection. After esophagectomy pathologic evaluation revealed higher portion of complete pathologic response in the chemoradiation plus enoxaparin arm (64.8% vs. 62.5%, p=0.9). Table 2 shows the difference of responses between the two groups. One years DFS was higher among patients who received chemoradiation plus

enoxaparin (78.9% vs. 70%, p=0.5). Figure 2 shows Kaplan–Meier plots of estimated 3-year disease free survival.

3.3. Adverse effects

One patient in the chemoradiation plus enoxaparin arm was excluded from the study due to severe thrombocytopenia. Similarly, in the chemoradiation alone arm, one patients was excluded due to severe pancytopenia during the study. The proportion of patients experiencing any adverse event was relatively higher in the chemoradiation plus enoxaparin arm (9/37 vs. 2/37, p=0.02); however, all adverse effects in both groups were related to the chemotherapy (all of them were neutropenia, the grade 1-2 in case and control group was 15.6% and 3.1% and grade 3-4 was 10.8% and 3.1%, p=0.03). Except patients who were excluded, no other serious adverse events were reported.

4. Discussion

This study was undertaken to investigate the effects of adding enoxaparin to neoadjuvant chemoradiotherapy in esophageal cancer. In the intervention group, daily prescription of 40mg enoxaparin was added to chemoradiotherapy [EBRT: 50Gy/2Gy, once daily, five days in week concurrent with paclitaxel (50 mg/m2) +carboplatin (AUC=2) weekly)]. Altogether, results of present study showed that there was a non-significant trend to improvement in the clinical and pathologic response in patients who were treated with enoxaparin. In addition concurrent neoadjuvant chemoradiotherapy with enoxaparin led to an improvement of1y DFS and a 7 month increase in the median survival. Although the difference was nonsignificant.

Despite the several studies reporting antitumoral effects and survival advantages of anticoagulant treatment, and specifically LMWH, in patients with cancer such as breast (Mellor et al., 2007)

lung (Altinbas et al., 2004; Bobek et al., 2005) and high grade brain gliomas (Perry et al., 2010), these effects have not be investigated in esophageal cancer and the present study appears to be a novel one.

Early studies by Bell et all (Bell, 1978), DiPalma et all (DiPalma and McMichael, 1979), and Hilgard et al (Hilgard and Thornes, 1976) have shown the potential effects of heparin against angiogenesis, and thereby, against transplanted tumor tissue growth at cellular level, which caused early clinical trials about antitumoral effects of anticoagulant drugs, and resulted in three large trials Malignancy and Low Molecular Weight-Heparin Therapy (MALT) (Klerk et al., 2005), Fragmin Advanced Malignancy Outcome Study (FAMOUS) (Kakkar et al., 2004),and CLOT (Lee et al., 2005) between 2004 and 2005. These studies show that LMWHs causes an improvement in survival in patients with cancer, independent of their antithrombotic effect (Kakkar et al., 2004; Klerk et al., 2005; Lee et al., 2005).

In an earlier trial, the MALT, patients with advanced malignancy were randomly divided into two groups: placebo (n=154) and nadroparin (n=148) and treated for 6weeks. Enrolled patients in this study were mostly patients with adenocarcinoma, and most had breast, lung and/or colorectal cancers. In the 12 months follow-up, the HR for death was 0.75 (95% CI:0.59-0.96) and median survival in nadroparin and placebo were 8 and 6.6 month, respectively, significantly improved in treated patients with nadroparin (Lee et al., 2005). The efficacy of one year treatment with dalteparin was evaluated in the FAMOUS trial (Kakkar et al., 2004). As in MALT, most of participants of the FAMOUS suffered from advanced malignancy (stage III or IV), mostly breast, colorectal, and ovarian cancer. Results of Kaplan-Meier survival analysis did not show any survival improvement, after one, two and three year, although patients who survived at least 17 month, appeared to benefit from treatment with dalteparin (two and three

year survival 78% vs 55% and 60% vs 36% respectively, p=0.03)(9). This study, importantly, showed that survival benefit of dalteparin persisted even after discontinuation of the drugs that translate into possible antitumoral effects of LMWHs, independent from their antithrombotic effects. In the CLOT trial, over 600 patients with simultaneous cancer and DVT were treated with warfarin or dalteparin for about six month. In non-metastatic patients, use of LMWHs was associated with improved survival and decreased mortality rate [mortality rate in LMWH and warfarin groups was 20% and 36%, respectively (HR 0.50; 95% CI: 0.27-0.95; p=0.03)]. In patients with metastatic disease, there were no survival benefits (HR 1.1; 95% CI: 0.87-1.4; p=0.46) (Lee et al., 2005).

Nonetheless previous studies reported survival benefits of warfarin (Schulman and Lindmarker, 2000; Zacharski et al., 1981) and decreased in incidence of new cancers in patients with cancer (Tagalakis et al., 2007) but one meta-analysis that compared mortality following treatment with warfarin or LMWHs in 11 clinical trials, only LMWHs were associated with improved survival (RR 0.877;95% CI:0.789-0.975;p=0.015); however, this effect was not observed with warfarin (RR 0.942;95% CI: 0.854-1.040; p=0.239) (Kuderer et al., 2007). A recent systematic review show that using heparin in cancer patients was associated with improved survival (HR 0.77, 95% CI 0.65-0.91) (specifically in patients with early stage disease) without increasing the risk of hemorrhagic events (RR 1.78,95% CI0.73-4.38) (Akl et al., 2007). It seems that LMWHs have potential role in improvement of survival in a subgroup of patients with cancer, although more investigations are needed to detect the rate and mechanisms of their efficacy according to the type, site and stage of disease.

There are controversies regarding possible effects of LMWHs in the management of cancerous patients (with goal of improvement of survival). For example, two relatively/quite large clinical

trials investigation the antitumoral effects of the ultra-low molecular weight heparins, Semuloparin (Agnelli et al., 2012) and nadroparin (Agnelli et al., 2009), did not show any survival benefit compared to LMWH. It is noteworthy that the major problem of most of previous research was heterogeneity in patients (Akl et al., 2007). Some studies in homogeneous patients with solid tumors like breast (Haas et al., 2012), malignant glioma (Perry et al., 2010) and lung cancer (Macbeth et al., 2015), were also, unable to show clinical benefits from adding LMWH to the standard treatment of cancerous patients.

Despite these controversies about the survival advantages of LMWH in solid tumors, in two recent review studies by Akl et al (2014) (Akl et al., 2014) and Zhang et al (2016) (Zhang et al., 2016), the authors concluded that LMWHs have clinical benefits in survival improvement of patients with cancer and considering the low risk of hemorrhagic events associated with combination of LMWHs and other chemotherapeutic agents, future investigation in evaluating different LMWH efficacies on different pathologies is warrant. However, it should be noted that according to current evidence, no guidelines recommend the use of LMWHs in order to improve survival of cancer patients and this indication is off-label (Geerts et al., 2008; Lyman et al., 2007; Mousa, 2006; National Comprehensive Cancer Network, 2011).

Clinical trials investigation the benefits of different types of LMWHs in the treatment of solid tumors, several studies have looked at the underling molecular mechanisms of these drugs. Each LMWH has special structural profile which causes unique pharmacokinetics and pharmacodynamics related to the drug structural differences between LMWHs including molecular weight, size, end component, carboxyl to sulfate ratio, and adhesive part of anti-Xa ratio, that have an effect on their biological activity (Fareed et al., 2004; Jeske et al., 2008).

Currently, there are ongoing studies in order to detecting/distinguishing antitumoral and antimetastatic features of these drugs (Casu et al., 2007).

It appears that the improvement in survival of patients with cancer, when using LMWHs, is not merely due to a reduction in the risk of DVT and pulmonary embolism, but due to the anti-tumoral effects of these drugs. LMWHs influence cancer cell growth directly by interfering in the coagulation cascade or hypothetical mechanisms (including anti-proliferative functions) (Balzarotti et al., 2006), and indirectly by modulatory effects of mucopolysaccharides chains on cell signaling and the cell/environment interactions, and on enzymes and cell-signaling molecules (Mousa, 2006). Anti-tumoral effects of low molecular weight heparin is mainly through inhibition of the interactions between P selectin of platelet and P selectin ligands on the surface of tumor cell that causing formation of the tumor cell-platelet complex. Via their P-selectin, platelets interact with both the endothelium and tumor cells helping their extravasations to the vascular compartment (figure 3) (Javadinia et al., 2018).

Furthermore, glycosaminoglycans or mucopolysaccharides chains cause the anti-metastatic effects of these drugs by interfering in the P-selectin mediated tumor metastasis pathway, and this is confirmed by lack of anti-metastatic effects in fundaparinox (a LMWH without mucopolysaccharides chain) (Stevenson et al., 2005). Beside the anti-tumoral effects of LMWHs, these drugs also possess anti-angiogenic effects as well (Folkman et al., 1983), and cause dose dependent anti-angiogenesis effects by simulating the endothelial tissue inhibitory pathways, and leading to inhibitory effects on tissue factors (Coughlin, 2005; Mitroulis et al., 2011; Norrby, 2006). As well as the anti-tumoral effects of heparins through inhibition of growth and angiogenesis, they can also directly affect the immune system by inhibitory effects on extravasation of leucocytes and the complement system, or increasing the sensitivity of tumoral

cells to immunological attack (Coughlin, 2005; Handa et al., 1991; Itoh et al., 1995; Mitroulis et al., 2011; Mousa and Mohamed, 2004; Norrby, 2006; Stevenson et al., 2005), therefore influence the anti-tumoral immunologic processes (Zhang et al., 2016).

Overall, the results of present study indicate that integration of enoxaparin into chemoradiation protocol is safe and tolerable. However, higher probability of neutropenia was reported in the patients treated with enoxaparin (no febrile neutropenia was reported in both groups). There are reports showing that neutropenia was greater in patients treated with chemotherapy +LMWH compared with those treated with chemotherapy alone (Altinbas et al., 2004). Also, the data are conflicting and Kucukoner et al. (2012) observed that neutropenia occurred more in patients treated with chemoradiation alone compare to patients received LMWH as well (Kucukoner et al., 2012).

The treatment of patients with esophageal SCC remains controversial since most previous investigations that determine our current practice, are fully adopted from the treatment approaches of developed countries that current effort try to overcome these uncertainties. A further positive aspect of our study was the evaluation of LMWHs anti-tumoral properties in a relatively homogenous population (with respect to disease type and histology). Limitations of our study were the lack of chemotherapy in a number of intervention group, which is associated with a reduction in the chemosensitizer effects of the indicated drugs and failure in monitoring of anti-Xa levels during the study. Considering the various effects of LMWHs in the inhibition of several pathways of tumors cell growth and metastasis and a possible a direct antitumor effect of LMWHs (e.g. a direct interference with components of the coagulation cascade), it is suggested that further studies should be conducted with higher sample size and considering the anti-Xa levels monitoring during the study and pattern of recurrence and metastasis in patients. Also,

regarding that cellular investigations indicate to dose dependent anti-tumoral properties of LMWHs, it is suggested that different dosages of these combinations should be experienced/tested in future researches.

5. Conclusion

Overall, the results of this study showed that the clinical and pathological response of squamous cell carcinoma of esophagus to the neoadjuvant chemoradiation was improved by the addition of enoxaparin to the treatment, although the difference was not significant. Also, there was an insignificant improvement in one year disease free survival of chemoradiotherapy patients receiving enoxaparin. Data from our study indicate that concurrent enoxaparin with radiotherapy and weekly paclitaxel/carboplatin chemotherapy was associated with minimal toxicity. The effects of LMWHs on survival of cancer patients, is probably due to a combination of direct anti-tumoral effects, antiangiogenic and immunomodulatory effects, beside indirect effects on the coagulation system. Most of these direct and indirect effects may have clinical efficacy in the treatment of SCC and gasteroesophageal adenocarcinoma, although the current data on this are contradictory and the observed benefits have been mostly from cellular and in-vitro investigations. Considering that treatment with LMWHs has few side effects, it is recommended that efforts to define the mechanisms of this group of these drugs in affecting tumor growth in the cellular level, and also clinical trials on the benefits of the anticoagulant and anti-tumoral effects, should be continued. However, it must be noted that the new generation of LMWHs lack the oligosaccharide segment and thus part of the antitumoral effects of these drugs may be limited.

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Conflicts of Interest

The authors declare that there were no conflicts of interest.

References

Agnelli G, George DJ, Kakkar AK, Fisher W, Lassen MR, Mismetti P, Mouret P, Chaudhari U, Lawson F, Turpie AG. 2012. Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer. New England Journal of Medicine 366(7):601-609.

Agnelli G, Gussoni G, Bianchini C, Verso M, Mandalà M, Cavanna L, Barni S, Labianca R, Buzzi F, Scambia G. 2009. Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. The lancet oncology 10(10):943-949.

Akl EA, Kahale LA, Ballout RA, Barba M, Yosuico VE, van Doormaal FF, Middeldorp S, Bryant A, Schunemann H. 2014. Parenteral anticoagulation in ambulatory patients with cancer. Cochrane Database Syst Rev(12):Cd006652.

Akl EA, van Doormaal FF, Barba M, Kamath G, Kim SY, Kuipers S, Middeldorp S, Yosuico V, Dickinson HO, Schünemann H. 2007. Parenteral anticoagulation for prolonging survival in patients with cancer who have no other indication for anticoagulation. Cochrane Database Syst Rev 3.

Allum WH, Stenning SP, Bancewicz J, Clark PI, Langley RE. 2009. Long-term results of a randomized trial of surgery with or without preoperative chemotherapy in esophageal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27(30):5062-5067.

Altinbas M, Coskun HS, Er O, Ozkan M, Eser B, Unal A, Cetin M, Soyuer S. 2004. A randomized clinical trial of combination chemotherapy with and without low-molecular-weight heparin in small cell lung cancer. Journal of thrombosis and haemostasis : JTH 2(8):1266-1271.

Anvari K, Pakdel AF, Memar B, Parsamanesh R, Ejlalzadeh SM, Javadinia SA. 2017. The prevalence and expression pattern of melanoma-associated antigen 1 in esophageal squamous cell carcinoma: a historical cohort study. Electronic Physician 9(2):3756-3763.

Balzarotti M, Fontana F, Marras C, Boiardi A, Croci D, Ciusani E, Salmaggi A. 2006. In vitro study of low molecular weight heparin effect on cell growth and cell invasion in primary cell cultures of high-grade gliomas. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics 16(5):245-250.

Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Ruschoff J, Kang YK. 2010. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet (London, England) 376(9742):687-697.

Bell RG. 1978. Vitamin K and chemical carcinogenesis. Lancet (London, England) 1(8074):1161.

Bobek V, Boubelik M, Fišerová A, L'uptovcova M, Vannucci L, Kacprzak G, Kolodzej J, Majewski AM, Hoffman R. 2005. Anticoagulant drugs increase natural killer cell activity in lung cancer. Lung Cancer 47(2):215-223.

Casu B, Vlodavsky I, Sanderson RD. 2007. Non-anticoagulant heparins and inhibition of cancer. Pathophysiology of haemostasis and thrombosis 36(3-4):195-203.

Coughlin SR. 2005. Protease-activated receptors in hemostasis, thrombosis and vascular biology. Journal of Thrombosis and Haemostasis 3(8):1800-1814.

Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, Participants MT. 2006. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355(1):11-20.

De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, Pierannunzio D, Trama A, Visser O, Brenner H, Ardanaz E, Bielska-Lasota M, Engholm G, Nennecke A, Siesling S, Berrino F, Capocaccia R. 2014.

Cancer survival in Europe 1999-2007 by country and age: results of EUROCARE--5-a population-based study. The Lancet Oncology 15(1):23-34.

DiPalma JA, McMichael R. 1979. The interaction of vitamins with cancer chemotherapy. CA: a cancer journal for clinicians 29(5):280-286.

Fareed J, Ma Q, Florian M, Maddineni J, Iqbal O, Hoppensteadt DA, Bick RL. Differentiation of low-molecular-weight heparins: impact on the future of the management of thrombosis; 2004. Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. p 89-104.

Folkman J, Langer R, Linhardt RJ, Haudenschild C, Taylor S. 1983. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science 221(4612):719-725.

Geerts WH, Bergqvist D, Pineo GF, Heit JA, Samama CM, Lassen MR, Colwell CW. 2008. Prevention of venous thromboembolism: American College of Chest Physicians evidence-based clinical practice guidelines. Chest journal 133(6_suppl):381S-453S.

Haas SK, Freund M, Heigener D, Heilmann L, Kemkes-Matthes B, Tempelhoff G-Fv, Melzer N, Kakkar AK. 2012. Low-molecular-weight heparin versus placebo for the prevention of venous thromboembolism in metastatic breast cancer or stage III/IV lung cancer. Clinical and Applied Thrombosis/Hemostasis 18(2):159-165.

Handa K, Nudelman ED, Stroud MR, Shiozawa T, Hakomori S-i. 1991. Selectin GMP-140 (CD62; PADGEM) binds to sialosyl-Lea and sialosyl-Lex, and sulfated glycans modulate this binding. Biochemical and biophysical research communications 181(3):1223-1230.

Hilgard P, Thornes R. 1976. Anticoagulants in the treatment of cancer. European Journal of Cancer (1965) 12(10):755-762.

Itoh K, Nakao A, Kishimoto W, Takagi H. 1995. Heparin effects on superoxide production by neutrophils. European surgical research 27(3):184-188.

Javadinia SA, Gholami A, Joudi Mashhad M, Ferns GA, Shahidsales S, Avan A, Taghizadeh Kermani A. 2018. Anti-tumoral effects of low molecular weight heparins: A focus on the treatment of esophageal cancer. Journal of Cellular Physiology 0(0):1-7.

Jeske WP, Walenga JM, Hoppensteadt DA, Vandenberg C, Brubaker A, Adiguzel C, Bakhos M, Fareed J. Differentiating low-molecular-weight heparins based on chemical, biological, and pharmacologic properties: implications for the development of generic versions of low-molecular-weight heparins; 2008. © Thieme Medical Publishers. p 074-085.

Kakkar AK, Levine MN, Kadziola Z, Lemoine NR, Low V, Patel HK, Rustin G, Thomas M, Quigley M, Williamson RC. 2004. Low molecular weight heparin, therapy with dalteparin, and survival in advanced cancer: the fragmin advanced malignancy outcome study (FAMOUS). Journal of Clinical Oncology 22(10):1944-1948.

Klerk CP, Smorenburg SM, Otten H-M, Lensing AW, Prins MH, Piovella F, Prandoni P, Bos MM, Richel DJ, van Tienhoven G. 2005. The effect of low molecular weight heparin on survival in patients with advanced malignancy. Journal of clinical oncology 23(10):2130-2135.

Kucukoner M, Isikdogan A, Kaplan MA, Inal A, Zinciroglu S, Cit M, Cil T, Karadayi B, Dirier A, Yildiz I. 2012. Can LMWH improve the outcome of patients with inoperable stage III non-small cell lung cancer? Contemporary Oncology 16(5):416-419.

Kuderer NM, Khorana AA, Lyman GH, Francis CW. 2007. A meta-analysis and systematic review of the efficacy and safety of anticoagulants as cancer treatment. Cancer 110(5):1149-1161.

Lee AY, Rickles FR, Julian JA, Gent M, Baker RI, Bowden C, Kakkar AK, Prins M, Levine MN. 2005. Randomized comparison of low molecular weight heparin and coumarin derivatives on the survival of patients with cancer and venous thromboembolism. Journal of clinical oncology 23(10):2123-2129.

Lyman GH, Khorana AA, Falanga A, Clarke-Pearson D, Flowers C, Jahanzeb M, Kakkar A, Kuderer NM, Levine MN, Liebman H. 2007. American Society of Clinical Oncology guideline: recommendations for venous thromboembolism prophylaxis and treatment in patients with cancer. Journal of clinical oncology 25(34):5490-5505.

Macbeth F, Noble S, Evans J, Ahmed S, Cohen D, Hood K, Knoyle D, Linnane S, Longo M, Moore B. 2015. Randomized phase III trial of standard therapy plus low molecular weight heparin in patients with lung cancer: FRAGMATIC trial. Journal of Clinical Oncology 34(5):488-494.

Medical Research Council Oesophageal Cancer Working Group. 2002. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet (London, England) 359(9319):1727-1733.

Mellor P, Harvey J, Murphy K, Pye D, O'Boyle G, Lennard T, Kirby J, Ali S. 2007. Modulatory effects of heparin and short-length oligosaccharides of heparin on the metastasis and growth of LMD MDA-MB 231 breast cancer cells in vivo. British journal of cancer 97(6):761-768.

Mitroulis I, Kambas K, Anyfanti P, Doumas M, Ritis K. 2011. The multivalent activity of the tissue factor–thrombin pathway in thrombotic and non-thrombotic disorders as a target for therapeutic intervention. Expert opinion on therapeutic targets 15(1):75-89.

Mousa SA. 2006. Role of current and emerging antithrombotics in thrombosis and cancer. Drugs of Today 42(5):331.

Mousa SA, Mohamed S. 2004. Inhibition of endothelial cell tube formation by the low molecular weight heparin, tinzaparin, is mediated by tissue factor pathway inhibitor. Thrombosis and haemostasis 92(3):627-633.

National Comprehensive Cancer Network. 2011. NCCN Clinical Practice Guidelines in Oncology. Venous Thromboembolic Disease, V2. 2008.

Norrby K. 2006. Low-molecular-weight heparins and angiogenesis. Apmis 114(2):79-102.

Perry J, Julian J, Laperriere N, Geerts W, Agnelli G, Rogers L, Malkin M, Sawaya R, Baker R, Falanga A. 2010. PRODIGE: a randomized placebo-controlled trial of dalteparin low-molecular-weight heparin thromboprophylaxis in patients with newly diagnosed malignant glioma. Journal of Thrombosis and Haemostasis 8(9):1959-1965.

Posner MC, Minsky BD, Ilson DH. 2015. Cancer of the Esophaguse. In: DeVita VT, Lawrence TS, Rosenberg SA, editors. DeVita, Hellma, and Rosenberg's Cancers: Principles & Practice of Oncology. 10th ed. Philadelphia: Lippincott Williams & Wilkins (LWW). p 574-612.

Schulman S, Lindmarker P. 2000. Incidence of cancer after prophylaxis with warfarin against recurrent venous thromboembolism. N Engl J Med 2000(342):1953-1958.

Shapiro J, van Lanschot JJB, Hulshof M, van Hagen P, van Berge Henegouwen MI, Wijnhoven BPL, van Laarhoven HWM, Nieuwenhuijzen GAP, Hospers GAP, Bonenkamp JJ, Cuesta MA, Blaisse RJB, Busch ORC, Ten Kate FJW, Creemers GM, Punt CJA, Plukker JTM, Verheul HMW, Bilgen EJS, van Dekken H, van der Sangen MJC, Rozema T, Biermann K, Beukema JC, Piet AHM, van Rij CM, Reinders JG, Tilanus HW, Steyerberg EW, van der Gaast A. 2015. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. The Lancet Oncology 16(9):1090-1098.

Siegel RL, Miller KD, Jemal A. 2016. Cancer statistics, 2016. CA Cancer J Clin 66(1):7-30.

Stevenson JL, Choi SH, Varki A. 2005. Differential metastasis inhibition by clinically relevant levels of heparins—correlation with selectin inhibition, not antithrombotic activity. Clinical Cancer Research 11(19):7003-7011.

Tagalakis V, Tamim H, Blostein M, Collet J-P, Hanley JA, Kahn SR. 2007. Use of warfarin and risk of urogenital cancer: a population-based, nested case-control study. The lancet oncology 8(5):395-402.

Ychou M, Boige V, Pignon JP, Conroy T, Bouche O, Lebreton G, Ducourtieux M, Bedenne L, Fabre JM, Saint-Aubert B, Geneve J, Lasser P, Rougier P. 2011. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29(13):1715-1721.

Zacharski LR, Henderson WG, Rickles FR, Forman WB, Cornell C, Forcier RJ, Edwards R, Headley E, Kim S-H, O'Donnell JR. 1981. Effect of warfarin on survival in small cell carcinoma of the lung: Veterans Administration Study No. 75. Jama 245(8):831-835.

Zhang N, Lou W, Ji F, Qiu L, Tsang BK, Di W. 2016. Low molecular weight heparin and cancer survival: clinical trials and experimental mechanisms. Journal of cancer research and clinical oncology 142(8):1807-1816.

Table 1. Patient and tumor characteristics per treatment group

		CRT+enoxaparin	CRT alone	*P* value
Variables		Frequency (%)	Frequency (%)	
Age (years)†	60≥	13 (35.1)	8 (25)	*p*=0.4
	61<	24 (64.9)	24 (75)	
Gender†	Female	18 (48.7)	15 (46.8)	*p*=0.8
	Male	19 (51.3)	17 (53.2)	
ECOG PR†	0-I	37 (100)	32 (100)	-
	II	0 (0)	0 (0)	
Tumor location‡	Upper thoracic	1 (2.7)	2 (6.3)	*p*=0.3
	Middle thoracic	21 (56.8)	13 (40.6)	
	Lowe thoracic	15 (40.5)	17 (53.1)	
Tumor grade †	I-II	29 (78.4)	25 (78.1)	*p*=0.8
	III-IV	8 (21.6)	7 (21.9)	

CRT: chemoradiation, ECOG PR: Eastern Cooperative Oncology Group (ECOG) performance status score, †Chi-Square test revealed no significant difference, ‡Fisher's exact test revealed no significant difference.

Table 2. The difference of clinical and pathologic responses between two groups

		CRT+enoxaparin	CRT alone	*P* value
Variables		Frequency (%)	Frequency (%)	
Clinical response[‡]	CCR	14 (38.9)	11 (35.5)	*p*=0.5
	SCR	21 (58.3)	17 (54.8)	
	PCR	1 (2.8)	3 (9.7)	
Classification of clinical response[†]	CCR	14 (38.9)	11 (35.5)	*p*=0.8
	RM	22 (61.1)	20 (64.5)	
Pathologic response[†]	pCR	24 (64.8)	20 (62.5)	-
	PD	13 (35.2)	12 (34.8)	
R staging[†]	R0 res	37 (100)	32 (100)	-
	R1 res	0 (0)	0 (0)	
	R2 res	0 (0)	0 (0)	

CCR: complete clinical response, SCR: significant clinical response, PCR: poor clinical response, pCR: pathologic complete response, PD: persistent disease. RM: residual mass, res: resection. Classification of clinical response based on CCR vs SCR+PCR. [†]Chi-Square test revealed no significant difference, [‡]Fisher's exact test revealed no significant difference.

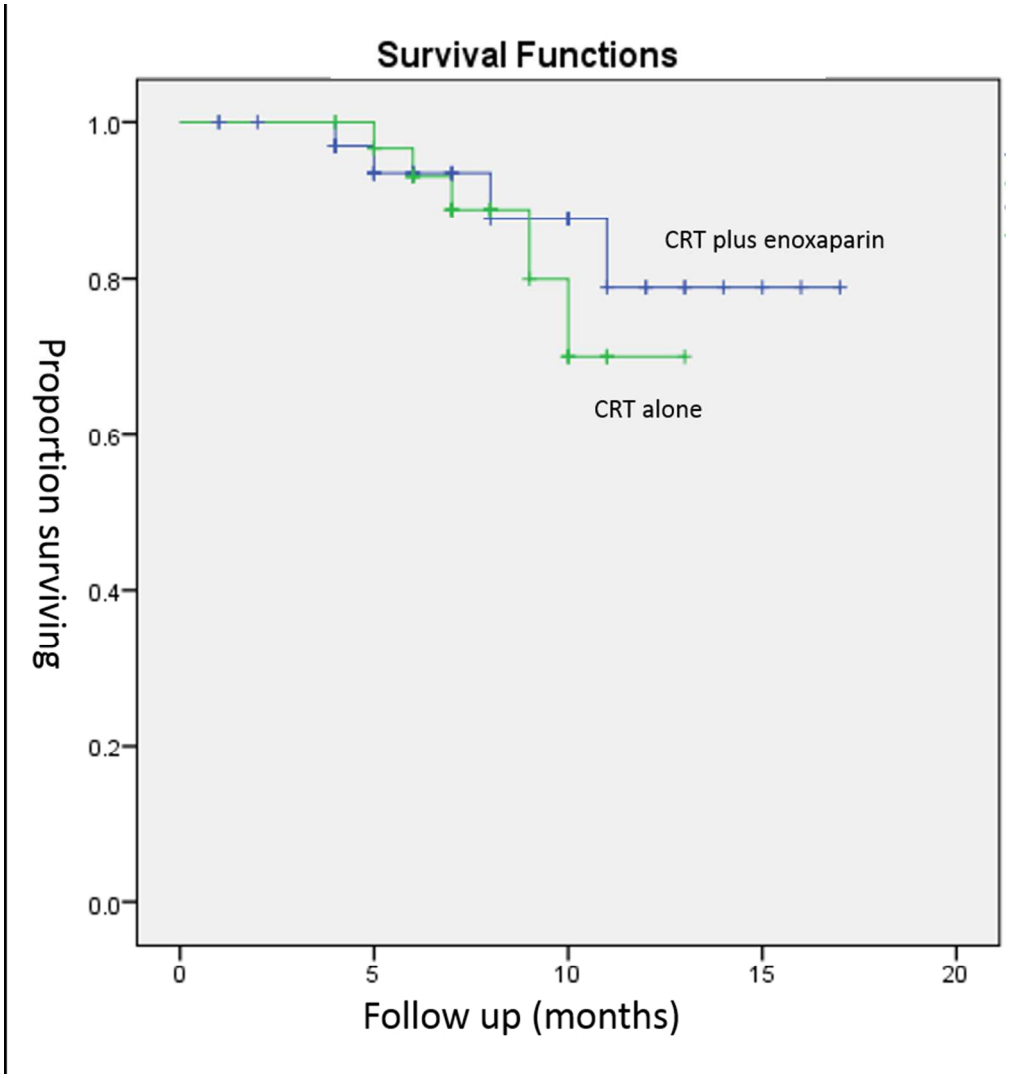

Figure 2. Kaplan–Meier Plots of Estimated 1-Year Disease Free Survival

157x168mm (150 x 150 DPI)

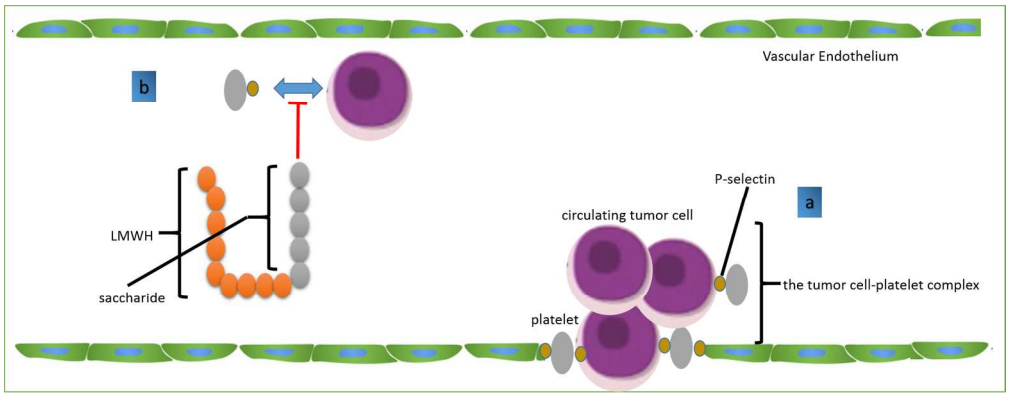

Figure 3: Anti-tumoral effects of low molecular weight heparin. a) Through the interactions between P selectin of platelet and P selectin ligands on the surface of tumor cell, the tumor cell-platelet complex is formed and causes dissemination. Via their P-selectin, platelets interact with both the endothelium and tumor cells helping their extravasations to the vascular compartment. b) LMWHs block the interaction between cancer cells and platelet and prevent the formation of tumor cell-platelet complex resulting in inhibition of metastasis.

322x124mm (150 x 150 DPI)